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Protein kinase C beta (PKC beta): normal functions and diseases.
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Nakashima S.
Protein kinase C alpha (PKC alpha): regulation and biological function.
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Protein Kinase Cγ (PKCγ): Function of Neuron Specific Isotype

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The gamma isotype of protein kinase C (PKCγ) is a member of the classical PKC (cPKC) subfamily which is activated by Ca^{2+} and diacylglycerol in the presence of phosphatidylserine. Physiologically, PKCγ is activated by a mechanism coupled with receptor-mediated breakdown of inositol phospholipid as other cPKC isotypes such as PKCα and PKCβ. PKCγ is expressed solely in the brain and spinal cord and its localization is restricted to neurons, while PKCα and PKCβ are expressed in many tissues in addition to the brain. Within the brain, PKCγ is the most abundant in the cerebellum, hippocampus and cerebral cortex, where the existence of neuronal plasticity has been demonstrated. Pharmacological and electrophysiological studies have shown that several neuronal functions, including long term potentiation (LTP) and long term depression (LTD), specifically require PKCγ. Generation of mice deficient in PKCγ provided more information regarding the physiological functions of this isotype. PKCγ deficient mice (i) have modified long term potentiation (LTP) in hippocampus, (ii) exhibit mild deficits in spatial and contextual learning (iii) exhibit impaired motor coordination due to persistent multiple innervations of climbing fibers on Purkinje cells, (iv) show attenuation of opioid receptor activation, and (v) show decreased effects of ethanol on type A of γ-aminobutyric acid (GABA) receptor. Furthermore, a point mutation in the PKCγ gene may contribute to retinitis pigmentosa and Parkinsonian syndrome. This article reviews the specific functions of this neuron-specific isotype of PKC in neuronal signal transduction.

Key words: GABA receptor, knockout mice, long term depression, long term potentiation, Parkinson disease.

Overview

PKCγ is a member of the classical PKCs (cPKC) which was first isolated as one of more than 10 PKC cDNAs from a brain cDNA library (*1*). The cPKCs, PKCα, PKCβ, and PKCγ, are activated by diacylglycerol (DAG) and Ca^{2+} in the presence of phosphatydylserine (*2, 3*). PKCγ was separated from PKCα and PKCβ biochemically using hydroxyapatite column chromatography, and the enzymological properties of the three isotypes were compared (*4*). Enzymological properties of this neuron specific isozyme are similar to those of PKCα and PKCβ which are described elsewhere in this series of reviews. The elucidation of PKCγ-specific function was first approached by determining the localization of PKCγ within the central nervous system. PKCγ shows a unique neuronal distribution and intracellular localization in the brain (*5–7*) but the involvement of this PKC isotype in the specific neuronal function is still unclear. The generation of mice deficient in PKCγ in 1993 (*8, 9*) provided an invaluable tool for the detailed analysis of PKCγ-specific function. Although the appearance and behavior of the PKCγ-knockout mice are not obviously abnormal, the experiments testing fine physiological and behavioral responses revealed significant effects of deletion of this enzyme. In this review, we focus on the physiological functions of this isozyme and its involvement in etiology of diseases.

1. Genomic and protein structure

The cDNA for PKCγ was sequenced in 1986 with those of PKCα and PKCβ (*10, 11*), then the genomic structure of PKCγ and its chromosomal mapping were analysed. The human and mouse PKCγ genes are localized on chromosomes 17q13.4 and 7, respectively. The human PKCγ gene is found on the most distal part of the chromosome, suggesting that there might be a telomeric position effect modifying the gene's expression throughout the replicative lifespan of human cells. The PKCγ gene is approximately 24.4 kb long and composed of small 18 exons varing between 32 and 406 bp in size (*12*). The AUG translation initiation site for open reading flames of PKCγ is localized in exon 1 as other cPKCs. Deletions or translocations involving the chromosomal region of PKCγ are frequently associated with malignant diseases such as leukemia [The Cancer Genome anatomy Project: recurrent chromosome aberrations in cancer (http://cgap.nci.nih.gov/Chromosomes/RecurrentAberrations)], although the functional role of this neuron specific PKC isotype in cancer is unclear. The 5′-flanking region of mouse PKCγ gene lacks TATA and CAAT boxes but contains the binding sites of transcription factors, including AP2 and SP1 (*13*). The region responsible for neuron-specific expression of PKCγ remains to be clarified.

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Abbreviations: PKC, protein kinase C; LTP, long term potentiation; LTD, long term depression; DAG, diacylglycerol; GABA, γ-aminobutyric acid; RP, retinitis pigmentosa; mGluR1, metabotropic receptor 1; PLCβ4, phospholipase Cβ4.

PKCγ has C1 and C2 domains which bind DAG and Ca^{2+}, respectively (*14, 15*). Both second messengers are necessary for the activation of cPKCs and fatty acids or lysoPC further enhances the activity of cPKCs (*16, 17*). The C1 domain of PKCγ consists of two cystein-rich repeats (C1A and C1B), both of which bind DAG with high affinity (*18, 19*); all nPKC family members have a single high affinity DAG binding site in C1B region. The structure of the PKCα. PKCβ. and PKCγ C1 domains are similar, while the amino acid sequences of their C2 domains show quite low homology. The functional differences resulting from this low homology are not known, but PKCγ appears to have higher affinity to Ca^{2+} than the others in the presense of phosphatydylserine (*20*).

2. Localization

The neuron specific distribution of PKCγ is the most unique characteristics of this isotype. PKCγ mRNA is solely found in the brain and spinal cord; it has not been found in any other tissues (*1*). Immunocytochemistry using isotype-specfic antibodies showed that all members of cPKC are enriched in the brain but their distributions are distinctly different (*7*). Abundant expression of PKCγ in the hippocampal pyramidal cells and cerebellar Purkinje cells (*5*) has implicated it in the modulation of synaptic plasticity, including long term potentiation (LTP) (*21*) and long term depression (LTD) (*22*).

Developmentally, the expression of PKCγ is low at birth and increases progressively up to 2–3 weeks (*23, 24*). In contrast, a considerable amount of PKCα and PKCβ are expressed before birth in brain, suggesting that PKCγ is important for synaptic formation rather than for early neuronal development and that the deletion of PKCγ is unlikely to alter early development.

Under electron microscopy, PKCγ is localized in the cytoplasm of the soma including nucleus and dendrites including dendritic spines, axon and synaptic terminals (*25, 26*). This intracellular localization also differs from those of other cPKCs (*7*), suggesting that the association of PKC isotypes with their specific substrates in different intracellular compartment results in isotype specific function. However, live imaging studies using GFP (green fluorescent protein)-tagged PKCγ revealed a rapid cycling of this isozyme between the cytoplasm and plasma membrane in cells upon stimulation of G-protein coupled receptors or Ca^{+}-ionophore (*27, 28*). The dynamic movement of PKCγ in response to various stimuli indicates that PKCγ does not always exist in the cytoplasm but interacts with specific substrates in other subcellular compartments when activated. Furthermore, as PKC isotypes show isotype-specific translocation and distinct translocation depending on stimulus (*29, 30*), the various PKC translocation, in addition to the existence of multiple isotypes, is a basic molecular mechanism of multiple function of this enzyme.

3. PKCγ specific functions in nervous system

3-1. LTP. Among various neuronal functions which involve PKC activation, modulation of synaptic plasticity by PKC has attracted the attention of biochemists and neuroscientists alike. Many papers have implicated the involvement of PKC in LTP, a synaptic model of memory. Activation of PKC by phorbol esters potentiates a synaptic transmission which resembles LTP in hippocampal slices (*31–33*). Additionally, direct injection of PKC into the postsynaptic hippocampal pyramidal cells mimics LTP (*34*). Finally, PKC inhibitors prevents the induction of LTP (*35*). Thus, the activation of postsynaptic PKC appears to be necessary for the induction of LTP. As PKCγ is predominantly localized in the postsynaptic dendrites in the hippocampal pyramidal cells, the data are consistent with involvement of PKCγ in LTP.

The generation of PKCγ knockout mice was reported in 1993 by Abeliovich *et al.* (*8*). The PKCγ deficient mice are viable and their brain anatomy is normal when examined by light microscopy. Behaviors such as grooming, feeding and mating are also unimpaired, although the mutant mice moved with a mild ataxic gait. The authors first examined the effect of deletion of PKCγ on the expression of LTP in the CA1 region of the hippocampus (*8*). Although synaptic transmission evoked by stimulating hippocampal axons in PKCγ deficient mice are indistinguishable from the wild-type mice, in PKCγ deficient mice, LTP is rarely induced by the commonly used high frequency stimulation. However, after a low frequency stimulation is used to produce LTD, LTP can be elicited in the knockout mice, suggesting that PKCγ regulates LTP but is not necessary for the actual process of synaptic plasticity. In fact, the knockout mice can learn to carry out hippocampus-dependent tasks, although they exhibit mild deficits in spatial and contextual learning (*9*).

3-2. LTD. LTD, the use-dependent decrease in synaptic strength, is the opposite phenomenon of LTP (*22, 36*). The involvement of PKCγ in LTD induction is strongly suggested by reports which show that (i) LTD is blocked by PKC inhibitors (*37*), (ii) PKC activators such as phorbol ester induced depression of synaptic transmission (*37*), and (iii) PKCγ is the major PKC isotype in Purkinje cells (*5*). Thus, the deletion of PKCγ would be predicted to abolish LTD. However, LTD is fully inducible in the cerebellar slices of the mutant mice (*38*). It is noteworthy that a PKC inhibitor peptide (PKC19-36) completely blocks LTD in wild-type mice but does not abolish LTD in mutant mice (*37, 39*). This suggests that PKCγ plays a pivotal role in LTD in wild-type mice and that unknown kinases compensate for the PKCγ deficiency in the mutant mice. This compensation for the lack in PKCγ by other kinase(s) makes it difficult to elucidate the specific function of PKCγ in the mutant mice.

PKCγ deficient mice exhibit impaired motor coordination but are fully capable of discrete motor learning (*40*). In mature mutant mice, 40% of Purkinje cells are innervated by multiple climbing fibers. In wild-type mice, these multiple innervations are eliminated during the 3rd week after birth, resulting in one-to-one innervation between the Purkinje cells and climbing fibers. There are several reports of other knockout mice which exhibit this persistent multiple innervation of Purkinje cells by climbing fibers. Mice deficient in metabotropic receptor 1 (mGluR1) (*41*) or phospholipase Cβ4 (PLCβ4) (*42*) show a similar phenotype of multiple innervation and motor discoordination. Taken together, the results from these mutant mice suggest that an mGluR1-PLCβ-PKCγ signaling pathway in cerebellar Purkinje cells is involved in the elimination of climbing fibers and the observed motor discoordination is due to the persistent multiple innervation of Purkinje cells by climbing fibers.

3-3. Modulation of receptor function. PKCγ is also abundant in the dorsal horn of the spinal cord and has been suggested to be important in sensory signal processing including pain. Several studies have shown that activation of μ-opioid receptors in the spinal cord induce prolonged PKC translocation (*43*) and that inhibition of PKC prevents the development of antinociceptive tolerance to μ-opioid agonists (*44*). Of the 10 PKC isotypes, the following evidence for the involvement of PKCγ in signaling for pain has been reported: selective μ-opioid receptor agonists (i) increase the amount of membrane-associated PKCγ but not other PKC isotypes and (ii) desensitize μ-opioid receptor-mediated G-protein activation (*45, 46*).

PKCγ deficient mice have been used to demonstrate that activation of PKCγ is critical for the development of morphine induced reinforcing (*46*) and enhancement of nociceptive responses (*47*). In PKCγ deficient mice, μ-opioid receptor–mediated analgesia/antinociception is enhanced and functional μ-opioid receptors are protected from degradation by phosphorylation (*48*). Furthermore, PKCγ deficient mice failed to develop a neuropathic pain syndrome after partial nerve section (*49*). These findings suggest that PKCγ may contribute to the induction of the psychological dependence on morphine and the development of PKCγ specific inhibitor may enable us to alleviate pain by protecting the tolerance. It is also interesting that epinephrine-induced hyperalgesia is also attenuated in mice lacking PKCε, a presynaptic localized isotype of nPKC (*50*). Although both PKCγ and PKCε act at different levels of the neuraxis, both isotypes play a role in pain responses.

The modulation of $GABA_A$ receptors by PKC was also reported but the role of PKC in their function is controversial. The response of $GABA_A$ receptors expressed in Xenopus oocytes is inhibited by phorbol esters (*51*) and the mutation of a possible PKC phosphorylation site, Ser343, in the γ2L subunit of $GABA_A$ receptor reduced the effect of PKC activation (*52*). In contrast, the activity of $GABA_A$ receptors expressed in fibroblasts are enhanced by active PKC (*53*).

Ethanol and benzodiazepines are known to enhance the function of $GABA_A$ receptor. The mutation of Ser343 in the γ2L subunit prevented ethanol potentiation but not benzodiazepine potentiation (*54*). $GABA_A$ receptors isolated from brain membranes of PKCγ deficient mice, do not respond to ethanol, although the deletion of the PKCγ gene does not alter their response to muscimol, fluinitrazepam or pentobarbital (*55*). Behaviorally, the mutant mice also display reduced sensitivity to the acute effects of ethanol on righting reflex and body temperature, but show normal responses to flunitrazepam or pentobarbital. Mutant mice consume more ethanol and display decreased tolerance development to the sedative hyptonic and hypothermic effects of ethanol (*56, 57*), suggesting that these PKCγ null mice may be a suitable model for the study of alcoholism. Interestingly, PKCε also regulates the sensitivity of $GABA_A$ receptor to ethanol. In contrast to PKCγ mutant mice, PKCε deficient mice show supersentivity to allosteric activation by ethanol and fluinitrazepam and exhibit reduced ethanol self-administration (*58*). These findings suggest that two PKC isotypes, PKCγ and PKCε, modulate the sensitivity of $GABA_A$ receptors to ethanol but have opposite actions on $GABA_A$ receptor activity.

4. Mutation of PKCγ gene related to disease

The genomic mapping in patients was performed to search for functional PKC polymorphisms or mutations associated with familial genetic abnormalities. Linkage analysis revealed that a major locus of retinitis pigmentosa (RP) exists at chromosome position 19q, which includes the PKCγ gene. Additionally, a point mutation in PKCγ that segregates with RP was found in two RP families (*59*). The mutation is a C-A transversion, which substitutes a serine for an arginine residue at codon 659 in the catalytic domain of PKCγ. Although the physiological function of this isotype in retina is unknown, some immunocytochemical studies demonstrate the expression of PKCγ in the amacrine and ganglion cells but not in photoreceptor cells (*60*). The effect of Arg659 mutation on the kinase activity of PKCγ is unclear, but it is possible that this mutation affects PKCγ maturation, as Arg659 is present between two important residues for maturation, turn motif (Thr655) and hydrophobic motif (Thr674) (*61, 62*).

Another report suggests that PKCγ is a candidate gene for Parkinsonian syndrome. The AS/AGU rat is a spontaneously occurring mutation which exhibits altered behavior and and brain pathology resembling Parkinsonian syndrome. At an early age of AS/AGU rat, the extracellular dopamine levels are markedly decreased in the AS/AGU rat. Later, loss of dopaminergic cells in the substantia nigra and dysfunction of movement are evident (*63–65*). Positional cloning of the agu mutation showed that this locus is tightly linked to the PKCγ gene. A comparison of PKCγ cDNA sequence from AS/AGU rats and that from the parental AS strain revealed that the G to T exchange at nucleotide 841 alters a GAG (281Glu) codon to an inframe TAG stop codon (*66*). This new stop codon would truncate the PKCγ at 280 amino acids, resulting in expression of most of regulatory domain of PKCγ. The generation of knock-in mice expressing this truncated PKCγ instead of full length PKCγ would help the elucidation of the etiology of neurodegenerative diseases such as Parkinsonian syndrome. It is also noteworthy that LTP in the CA1 region of the hippocampus from AS/AGU rats is not altered (*67*), while LTP in PKCγ deficient mice is impaired under the conventional condition described above. Thus, it is possible that the regulatory domain of PKCγ in AS/AGU rats plays a role in LTP.

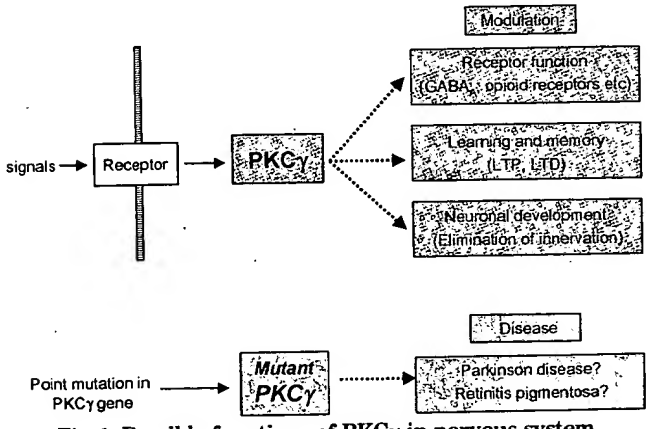

Fig. 1. Possible functions of PKCγ in nervous system.

Conclusion

Recent studies using PKCγ mutant mice have accumulated evidences for PKCγ specific functions in the nervous system (Fig. 1). However, as described above, PKCγ function could be compensated by other kinases, and neuronal network is subtly abnormal in the mutant mice. Further studies utilizing inducible knockouts of PKCγ are necessary to fully elucidate the neuronal functions of PKCγ.

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